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## TAXONOMIC AND MORPHOLOGICAL OBSERVATIONS ON *BOTRYCHIUM MULTIFIDUM* (OPHIOGLOSSACEAE)

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Once upon a time, Clausen (1938) recognized five subspecies of *Botrychium multifidum* (Gmel.) Rupr. although he acknowledged that they grade insensibly into one another especially where their ranges are confluent. For example, the North American range of ssp. *silatifolium* (Presl) Clausen is from the Sierra Nevada to the Pacific Northwest and across

to northeastern North America and overlaps with that of ssp. *coulteri* (Underw.) Clausen and ssp. *californicum* (Underw.) Clausen in the Sierra Nevada and with ssp. *multifidum* (ssp. "*typicum*" of Clausen) in British Columbia and the southern portion of northeastern North America. In contrast, ssp. *robustum* (Rupr.) Clausen is restricted to Alaska and the ranges of ssp. *robustum*, ssp. *multifidum*, and ssp. *californicum* do not overlap. Apparently, the basis for establishment of these subspecies was their seemingly plurimodal distribution as revealed by herbarium data. However, because Clausen's (1938) monograph was based primarily upon herbarium specimens, representative collections of variation within populations were probably not obtained. In fact, the concept of the plurimodal distribution of these subspecies has been recently questioned by Hitchcock et al. (1969).

The Ophioglossaceae have been considered unique [with the exception of *Cheilanthes* spp. and *Pteris* spp. (Knobloch, 1965)] in the Filicopsida in that adult leaves do not exhibit circinate vernation but rather have erect (conduplicate) or a somewhat bent vernation. However, some morphological treatments (Milde, 1858, 1869; Campbell, 1911, 1940; Troll, 1939; and Bierhorst, 1971) figure young leaves of some of the larger species of *Botrychium* in which the vernation appears to be circinate. Thus, an investigation of buds with emerging leaves of large plants of *B. multifidum* would be appropriate in elucidating the concept of vernation in this taxon.

Baas-Becking (1921) indicated that the roots of *B. multifidum* may be contractile, a feature that would be unique in the ferns. However, because he had only one shoot bearing only a few roots, he was unable to investigate this fully. Further documentation of this and the aforementioned features of taxonomy and vernation are the themes of this study.

#### MATERIALS AND METHODS

The following five Sierran populations of *B. multifidum* were observed, and collections of the subspecies circumscribed by Clausen (1938) as well as some intermediates have been deposited in the herbarium at the University of California at Davis: California, El Dorado Co., south of Lake Audrain, *Stevenson 365*, east side of Grass Lake at Luther Pass, *Stevenson 403-410*; Sierra Co., south side of Lower Sardine Lake, *Stevenson 367, 368*; Plumas Co., 0.8 km (0.5 mi) north of Grass Lake, *Stevenson 376-379*; Amador Co., north of Silver Lake near Kit Carson Lodge, *Stevenson 411-413*.

In addition, two smaller populations, one on the east side of Grass Lake, Plumas Co., and one at the west end of Lake Audrain were observed, but because of the paucity of individuals no collections were made.

Whole plants were killed and fixed in FPA. Some roots were removed at their point of attachment to the rhizome, embedded in Paraplast, and serially sectioned both longitudinally and transversely.

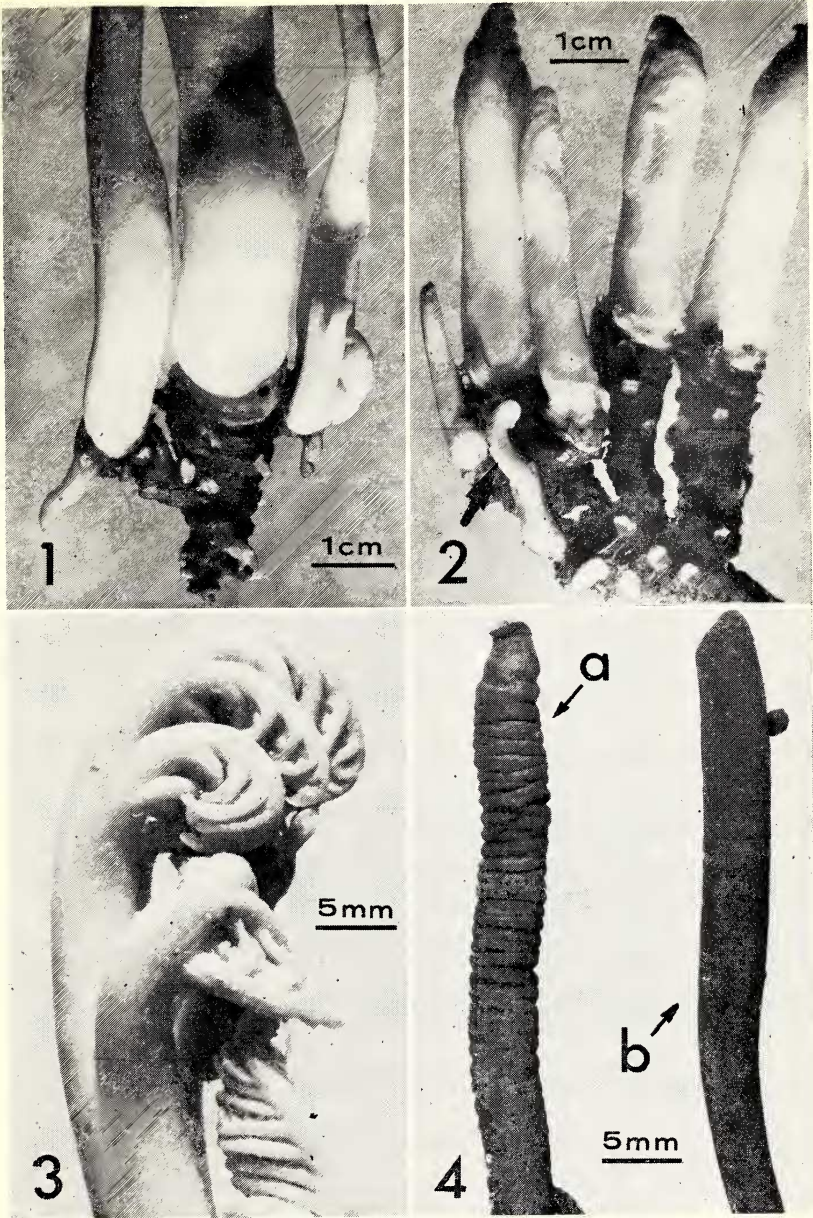
## OBSERVATIONS AND DISCUSSION

*Taxonomic considerations.* The morphological characteristics of the five subspecies of *B. multifidum* as circumscribed by Clausen (1938) are at best vague except for individual plants representing extreme conditions. For example, in the large population of several hundred plants at Luther Pass, each individual usually was an intermediate. However, the few extreme individuals provided at least one representative of each of the subspecies. Thus, this population contained some individuals that could be referred to *ssp. robustum* and *ssp. multifidum*; these would be far outside their ranges as recorded by Clausen (1938). This was also true for all other populations under observation.

It could be argued that the intermediates could well represent hybrids of sympatrically growing subspecies. However, an intense search for gametophytes in each population proved fruitless. This search included taking soil samples from depths down to 45 cm and a careful examination of the soil particles was made with the aid of a hand lens both before and after sifting the soil with various soil sieves. Moreover, gametophytes of *B. multifidum* have never been observed except for one illustration by Milde (1858), which was interpreted by Clausen (1938), but not by Milde, as a gametophyte with an attached sporophyte. Thus, it seems that sexual reproduction in this species is very rare. Furthermore, most of the large plants are probably very old. Plants of this taxon usually produce only one leaf per year but occasionally may produce two in a growing season. Thus, a conservative estimate of the age of an individual plant can be made by counting the leaf scars on the rhizome and dividing by two. For example, large rhizomes that had rotted away at their basal ends were collected that had more than 200 leaf scars; these plants were therefore more than 100 years old. Even the relatively few small plants (some with leaves composed of only three undivided pinnae) had minimum ages of 15 to 30 years. The paucity of young plants further supports the theory of infrequent sexual reproduction.

What can appear in the field as several closely placed individual plants may represent one copiously branched individual (figs. 1, 2). In some cases two (fig. 7a, b) or even three branches of the same plant were referable to different subspecies as circumscribed by Clausen (1938). In conclusion then, recognition of subspecific taxa in *B. multifidum* is questionable on numerous grounds, the most convincing being the presence of more than one of the previously described subspecies as branches of the same individual!

*Vernation.* One of the unique features of leaves of the Ophioglossales is their putative lack of circinate vernation as opposed to its generally consistent occurrence in both the Marattiales and Filicales. Leaf vernation in the Ophioglossales has been described as erect in the smaller forms and bent in the larger forms. With respect to the latter, the two segments, sterile and fertile, are curved towards each other with the sterile segment over-arching the fertile segment (fig. 3). In *B. multifidum*

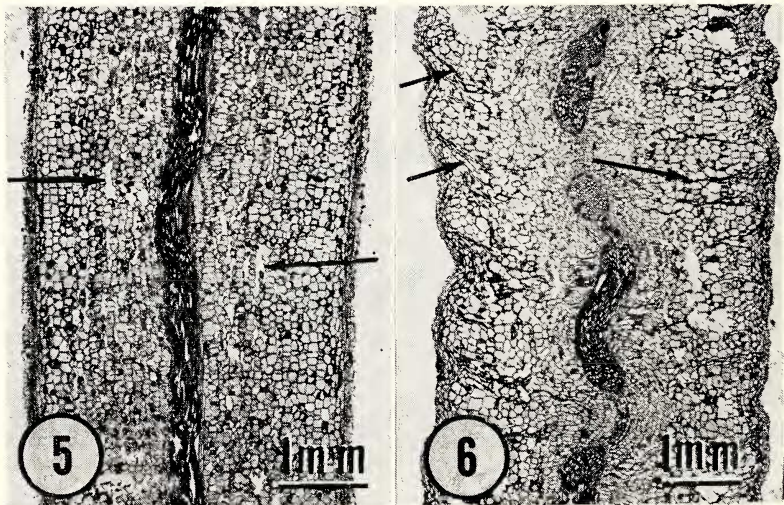


FIGS. 1-4. *Botrychium multifidum*. 1-2. Examples of highly branched individuals. Note circinate venation of small leaf (arrow) in 2. 3. Large leaf with circinate venation. 4. Older contracted root (a) and young uncontracted root (b).

(and throughout the Ophioglossales) each leaf primordium is enclosed in the stipular sheath of the next older leaf. The leaf and each of its sub-

sequent subdivisions grow by means of apical cells, as in the leaves of taxa placed in the Marattiales and Filicales. In vascular plants, growth of leaves by an apical cell is restricted to taxa with circinate vernation. Thus, it would not be inconsistent to expect circinate vernation of the large leaves of vigorously growing shoots of *B. multifidum*. An examination of emerging leaves of large plants of this species does indeed reveal circinate vernation of both fertile and sterile portions and their subdivisions (fig. 3). In fact, the degree of circination appears to increase with the increasing size of the mature leaves. In small plants bearing small leaves the leaves are merely bent or slightly circinate (fig. 2, arrow) as compared to larger leaves (fig. 3) of larger plants. Therefore, it seems reasonable to regard bent vernation of leaves of smaller species of *Botrychium* as diminutively circinate. Furthermore, occurrence of circinate vernation in the Ophioglossales makes their relationship with the Marattiales, and hence the Filicales, more tenable.

*Contractile roots.* The most obvious indication of contractile roots in *B. multifidum* is the wrinkled or corrugated appearance of the older roots (fig. 4a) as opposed to the smooth even appearance of young roots (fig. 4b). This wrinkled appearance usually extends from 2–4 cm along the root from its juncture with the rhizome (fig. 4a) and corresponds to the contracted portion of an individual root. The process of contraction was revealed by comparing serial sections of contracted roots with those of younger uncontracted roots. Lysigenous cavities form in the root cortex at somewhat regularly spaced intervals (fig. 5); then these cavities col-



FIGS. 5-6. Longisections of uncontracted and contracted roots of *Botrychium multifidum*. 5. Uncontracted root with developing lysigenous cavities (arrows) and a straight stele. 6. Contracted root with collapsed cavities (arrows) and undulating stele.

lapse (fig. 6) and the root contracts, resulting in the external wrinkled appearance. Also, the root stele, which is originally straight in longisection (fig. 5), becomes undulated or spiral so that only portions of it occur in any longisection (fig. 6). This method of root contraction is essentially the same as that reported for several species of *Oxalis* (Thoday, 1946; Davey, 1946).

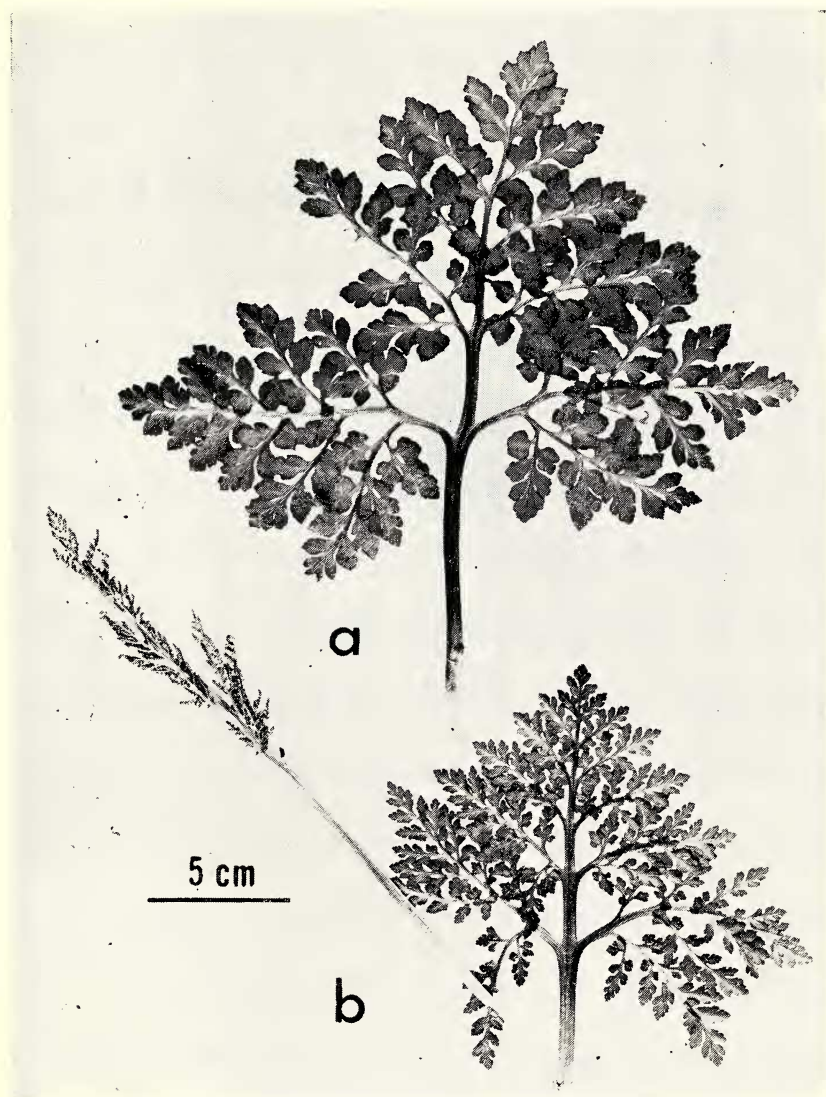


FIG. 7. A branched individual of *Botrychium multifidum* in which one shoot (a) has the characteristics of ssp. *californicum* and the other shoot (b) is intermediate between ssp. *coulteri* and ssp. *robustum*.

Occurrence of contractile roots in lower vascular plants seems to be limited to *B. multifidum*; however, there is some indication that they also occur in *B. dissectum*. Chamberlain (1920) described roots of this species as wrinkled and they are illustrated as such by Britton and Brown (1913). In addition, a cursory examination of herbarium specimens indicates that they are also present in *B. australe*, *B. jenmanii*, *B. alabamense*, and *B. underwoodianum*.

It is generally acknowledged that contractile roots either aid in anchoring plants in or on a substrate or in keeping them embedded in the soil. The latter appears to be the case in *B. multifidum* for the bud (the shoot apex and associated leaf primordia) is below the soil surface. As the shoot continues to grow and form new nodes, the vertical rhizome becomes longer as a result of the addition of the new growth increments. Eventually, the bud would arrive at the soil surface. However, the contractile roots that are produced at each node maintain the bud position below the surface. Thus, there is probably a balance established that maintains the bud at a more or less uniform distance beneath the soil surface.

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